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The Effect of Carbon Dioxide Concentration on Calcification in the Red Coralline Alga BOSSIELLA ORBIGNIANA

A. Dwight Smith

Abstract

THE EFFECT OF CARBON DIOXIDE CONCENTRATION

ON CALCIFICATION IN THE

RED CORALLINE ALGA BOSSIELLA ORBIGNIANA

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The relationship between various experimental concentrations of CO_2 and calcification was studied by measuring Ca-45 incorporation into the crystalline matrix. Air containing CO_2 at partial pressures (P_{CO_2}) of 0.04% to 5.5% was bubbled through synthetic sea water in incubation vessels. The resultant pH values ranged from 8.7 to 6.5. The relative concentrations of CO_2, HCO_3^-, and CO_3^{2-} were calculated from the measured pH-P_{CO_2} combinations. Calcification was correlated positively with HCO_3^- expressed as percent of total carbon. Maximum calcification of about one-third above normal occurred between 0.1% and 1.0% P_{CO_2}. At 0.26% P_{CO_2}, calcification increased as increments of $NaHCO_3$ in the range from 1 to 8 mM were added; this increase did not occur in the killed controls. The data suggest that calcification is controlled by a biological process that may be sensitive to pH and/or to the HCO_3^- concentration. The data also suggest that an increase in CO_2 over the present atmospheric level would significantly increase calcification in this marine alga.

LOMA LINDA UNIVERSITY

Graduate School

THE EFFECT OF CARBON DIOXIDE CONCENTRATION ON CALCIFICATION IN THE RED CORALLINE ALGA *BOSSIELLA ORBIGNIANA*

by

A. Dwight Smith

A Dissertation in Partial Fulfillment

of the Requirements for the Degree Doctor of Philosophy

in the Field of Biology

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Each person whose signature appears below certifies that this dissertation in his opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.


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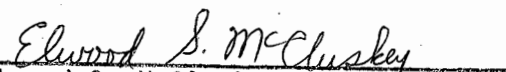
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To my Christian teachers, whose exemplary lives have encouraged me to pursue research that reveals the beauty in God's created works and glorifies His name, I dedicate this work.

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INTRODUCTION

Much concern has been expressed about the possible effects of increasing carbon dioxide concentration in the atmosphere. This build up of CO_2 is apparently due to increasing use of fossil fuels (Suess, 1973) and other human activities (Adams, Mantovani, and Lundell, 1977; Bolin, 1977). An increase in atmospheric CO_2 to ten times that of present levels is projected if all fossil fuel reserves are consumed (Plass, 1956; Coyne, 1975). According to Coyne (1975) plants and animals could survive in this atmosphere, but "the effect of the extra CO_2 on weather cycles and the buffering system in natural waters and thus on the concentration of pH dependent chemical species is a largely unanswered question." Plass (1956) states that an increase in atmospheric CO_2 to 50 times current levels would not change oceanic pH more than half a unit, a level that would be tolerated by marine organisms. The possible influence of carbon dioxide concentration on temperature has given support to the theory that changing CO_2 levels in the atmosphere determine long-term changes in the climate (Plass, 1956; Garrels, Lerman, Mackenzie, 1976). The enhancement of photosynthesis with increasing CO_2 concentrations (Bolin, 1977) supports the suggestion that the large amounts of organic carbon buried in the earth indicate greater environmental CO_2 levels in the past (Plass, 1956). Consistent with this is the common practice of elevating CO_2 levels in greenhouses to enhance crop production (Moss, 1976).

The effect of increasing atmospheric levels of CO_2 on calcification in marine organisms is not so well known. An optimum pH of 7.8 was found for the division rate of a calcifying unicellular coccolithophorid

using a medium buffered with the CO_2 - bicarbonate - carbonate system (Swift and Taylor, 1966). Calcification in Halimeda, an important green alga associated with tropical reefs (Goreau, 1963), was increased by bubbling 5% CO_2 in air through sea water (Stark, Almodovar and Krauss, 1969). Both calcification and photosynthesis rates in Halimeda were increased by increasing the CO_2 concentration through the use of several different buffers and increments of $NaHCO_3$ in acidified sea water (Borowitzka and Larkum, 1976b).

Changing CO_2 concentration might also have an effect on the upward rate of growth of reef structures, a subject that has been of interest to both biologists and geologists, especially since Darwin (1842) put forward his subsidence theory for the origin of atolls (Stoddart, 1969). Although most reefs are relatively shallow, Eniwetok Atoll is interpreted as being a reef that is over 1400 meters thick. If this is so, theories of reef development must account for an accumulation of this magnitude. That ecological factors were not always favorable for reef development is suggested by the occurrence of guyots (flat-topped seamounts) and drowned atolls (some of which are located very close to living reefs), commonly at a depth of 1000-2000 meters in the Indo-Pacific Basin (Menard, 1964).

Standard estimates for upward growth of coral reefs average about 1 cm yr^{-1} (Clausen, 1972). However, Smith (1973) in referring to the Eniwetok windward reef flat states that "there has been virtually no net $CaCO_3$ accumulation over the last several thousand years." A later paper, based on production rates of several shallow seaward reef flat environments (Smith and Kinsey, 1976), suggests "an upper limit of 3 to 5 mm yr^{-1} on the potential of modern reef communities to create a

significant vertical structure on a rising sea." This assumes present environmental conditions.

Because of its fundamental nature the process of calcification has received much attention; yet it is still not understood. The high degree of specificity and organization seen in the end products of calcification, such as the coccoliths of coccolithophorids, tests of foraminifers, and the crystalline matrix of molluscs and coralline algae, strongly suggest that enzymatic control and genetic regulation are involved; it is not just a simple precipitation event.

The differing light and dark calcification rates of corals (some of which calcify nine times faster in the light than in the dark (Goreau, 1959)) and of calcareous algae have been used as evidence that light or light produced substances are necessary for calcification (Goreau, 1963). However, there are claims that some photosynthetic organisms calcify faster in the dark (Goreau, 1963); and many non-photosynthetic organisms such as molluscs and vertebrates also carry on calcification, showing that the process does not have to be directly dependent on photosynthesis.

The photosynthetic inhibitor DCMU reversibly inhibits light-enhanced coral calcification without affecting other behavioral events (Vandermeulen, Davis, and Muscatine, 1972); and DCMU inhibits equally both photosynthesis and coccolith formation (Crenshaw, 1964). This is taken as evidence that light enhanced calcification is due largely to photosynthesis and not to some other photobiological event. However, Paasche (1964, 1965), using another coccolithophorid, found that CMU (another inhibitor of photosynthetic non-cyclic electron flow) was less inhibitory to coccolith formation than to carbon fixation or oxygen evolution.

Much of the work on algal calcification has involved coccolithophorids (see reviews of Wilbur, Colinvaux and Watabe, 1969; Paasche, 1968; and Darley, 1974) and the green alga Halimeda (Stark, Almodovar, and Krauss, 1969; Borowitzka and Larkum, 1976a, b, c, 1977a, b). A review in press (Borowitzka and Larkum, 1977b) summarizes much of the data in this field.

Crenshaw (1964) concluded that the calcium carbonate portion of the coccolith is formed by photosynthetic fixation of molecular carbon dioxide from bicarbonate assimilated from the medium. According to this view calcite is then precipitated on the preformed matrix which acts as a nucleation site. The equation would be $2HCO_3^- + Ca^{++} \rightleftharpoons CO_2 + CaCO_3 + H_2O$.

Both anatomical and physiological evidence are used by Borowitzka and Larkum (1974, 1976a, b, c and 1977a) to describe the calcification process in Halimeda. They suggest that before calcification begins chloroplasts must reach functional maturity; and the peripheral utricles must fuse together to create an intercellular space that is separated from the external sea water and could be maintained at a higher pH. Calcification begins in the (pilose) cell wall layer facing into this intercellular space. Only the initial nucleation of aragonite is associated with the pilose cell wall layer. Apparently later deposition of aragonite involves precipitation around existing aragonite needles.

Considerable information has been compiled regarding the chemistry of the sea (Harvey, 1957; Riley and Skirrow, 1966, 1975). The distribution of the carbonate system can be represented by $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$. The relative amount of each species depends on the pH, salinity and temperature of the sea water. In normal sea water about 1% of the total CO_2 exists as free CO_2, 9% as CO_3^{2-}, and 90% as HCO_3^-

(Steemann Nielsen, 1975). The concentration of CO_3^{2-} increases as pH increases and that of free CO_2 increases as pH decreases. At a pH slightly above 9, approximately half of the total carbon is HCO_3^- and half is CO_3^{2-}. A knowledge of the distribution of these carbon species is essential in determining their availability for photosynthesis, respiration, and calcification.

Either an open system or a closed system may be used for laboratory studies. In an open system CO_2 of known concentration is bubbled through the sea water until equilibrium is reached and then the distribution of carbon species determined by methods described by Park (1969). In the currently more popular closed system, sea water is acidified and then adjusted to the desired pH by adding $NaHCO_3$ or Na_2CO_3. Organic buffers are also used to keep the medium at a desired pH.

For this study I have used an open system because I feel that this more closely simulates the natural system when changing the concentration of CO_2 to represent atmospheric conditions that may have existed in the past or may exist in the future. The carbon dioxide-bicarbonate-carbonate system also acts as its own natural buffer system and I do not have to determine the effects of added artificial buffers, acid, or base. A coralline alga was chosen because (1) considerably less is known about it, (2) it is found locally, (3) Pearse (1972) has shown that it calcifies at rates similar to a closely related tropical reef species, and (4) Thomas (1976) has elucidated its response to temperature change.

MATERIALS AND METHODS

Collection and maintenance

Plants of the red coralline alga Bossiella orbigniana (Descaisne) Silva (1957) (Corallinaceae) (determined by H. W. Johansen) were collected subtidally between July 4, 1976 and March 21, 1977 near Treasure Island (= Goff Island) about 2 km south of Laguna Beach, California. The algae were transported in natural sea water of pH 8.1 to 8.3 and kept in an insulated container until needed. See Table 1.

Sea water system

Six plexiglass chambers measuring 12 cm x 14 cm x 9 cm high (Fig. 1) were each filled with one liter of synthetic sea water (Instant Ocean Synthetic Sea Salts, Aquarium Systems, Inc., Wickliffe, Ohio). After aeration with normal air, and without plants, the pH of this sea water was 8.3 to 8.4. The experimental set-up is pictured in Fig. 2.

Regulation of P_{CO_2} and pH measurement

The partial pressure of CO_2 (P_{CO_2}) in each chamber was adjusted by bubbling premixed CO_2 in air at a flow of 700–1000 ml min^{-1} into the chamber (except Experiment S-10, 1500 ml min^{-1}).

The tank P_{CO_2} levels as determined by mass spectrometer analysis expressed as percent of CO_2 in air were 0.04, 0.11, 0.23, 0.26, 0.39, 0.93, 1.05, 2.26 and 5.46. The relationship between pH and P_{CO_2} for our synthetic sea water was obtained by bubbling each gas mixture through sea water to which no plants had been added until the pH remained stable for several minutes. Since small changes in pH markedly affect calculations using pH and P_{CO_2} (Skirrow, 1975), a least-squares line (Fig. 3) was

fitted to the measured pH-P_{CO_2} points, and pH-P_{CO_2} combinations derived from the line were used for further calculations.

The pH for each chamber was measured with a Corning Model 112 pH meter equipped with a semi-micro combination electrode. Van Lab 7.00 and 10.00 buffers of Hepes and Tris buffers prepared in synthetic sea water were used to calibrate the electrodes. The beginning and ending pH values for each chamber are given in Table 2. The maximum variation (0.07 to 0.31 pH units) occurred when the 0.04% CO_2 in air was bubbled through the sea water. At other concentrations of CO_2 drift was nearly always negative and less than -0.15 pH units.

Distribution of carbon species in sea water

The relative concentrations of the various carbon species in synthetic sea water are shown in Fig. 4. The parameters used for the calculations are given in Table 3 and the measured pH-P_{CO_2} values were derived as explained in the previous section above. The assumption is made that the constants are pH independent (Pytkowitz, Ingle, and Mehrbach, 1974).

Factors that affect the CO_2 equilibrium in sea water

The addition of CO_2 or its salt ($NaHCO_3$, Na_2CO_3, etc.) disturbs the equilibrium of synthetic sea water, as seen in Figure 4, and changes the pH as well as the concentrations of the various carbon species. The bubbling of normal air or CO_2-enriched air hastens return to a new equilibrium. The new equilibrium will depend on:

(1) the P_{CO_2} of the prevailing atmosphere

(2) the degree of saturation of the sea water by CO_2; in my case, by the P_{CO_2} of the CO_2-enriched air, as well as by its flow rate (since it is an open system with plants)

(3) salinity of the new concentrations of the various ions, especially Na^+ and Mg^{++}

(4) the H^+ activity (measured as pH)

(5) the activity of the living organisms

(6) temperature

The solubility of $CaCO_3$ and the concentrations of H^+, CO_2, HCO_3^-, and CO_3^{2-} will all change each time a shift in equilibrium occurs.

Temperature and light

The room temperature remained below 13°C. Sea water temperature of 19 ±0.5°C (Thomas, 1976) was regulated using contact thermometers, two 100-watt immersion heaters per chamber, and thermoregulator relays. Two 110-watt high-output cool-white fluorescent lamps directly above the chambers provided 5mW cm^{-2} illumination at plant levels.

A comparison (Table 4) between plants held overnight in the dark before being used (Experiment L-1) and those collected on the day of the experiment (Experiment L-2) suggests that the previous history may affect rate of Ca-45 uptake and that experiments should be conducted as soon after collection as possible for optimum results (see also Roth, 1974; Thomas, 1976). Table 4 also suggests that the light intensity of 5mW cm^{-2} (see arrow) used for the standard experiments was not a limiting factor.

Preparation of plants and incubation conditions

Six plants were each divided into six parts and one part of each

plant assigned to each of the six experimental chambers using a random numbers table. In later experiments, a seventh plant was similarly distributed among the chambers after first being killed by keeping it in running tap water above 65°C for at least 30 minutes.

The time conditions for each experiment are given in Table 1. In order to minimize any circadian effects, most experiments were started between 12:00 and 14:10 on the same day that the plants were collected. Deviations from this are noted. A preincubation period of about one hour (see Table 1 for exceptions) was started after distributing the plants to the chambers, by turning on the lights, heaters, and gas flow. The incubation period commenced with the addition to each chamber of approximately 50–175 μC Ca-45 (with minimum specific activity of 9 to 32 mCi mg^{-1} $CaCl_2$) and lasted (in the light and the standard experiments) for two hours. In the experiments in which substances other than CO_2 were added, it was for one hour (except B3).

Post-incubation procedure

After incubation the algae were allowed to stand in tap water for several minutes (Table 1). Next the individual plant parts were each placed in a separate petri dish and covered with 6% sodium hypochlorite (commercial bleach) for three days. Kolesar (1973) found that calcareous algae continued to lose weight for a number of days after being placed in bleach, suggesting that the non-carbonate matrix is not quickly removed. When treated with 0.1N HCl after three days in the bleach, our algae tips showed no evidence of the ghost tissue that was found when non-bleached tips were so treated.

Radioassay method

Only terminal segments (tips) were assayed, because Pearse (1972) found them to be more active in Ca-45 incorporation than the older branch segments. Six tips were chosen from a plant part, placed on a planchet, and timed for 10,000 counts using a Nuclear-Chicago gas-flow detector. This gave six averages per treatment and six averages per plant for each experiment.

To correct for differences among chambers due to experimental errors, Ca-45 activity of the sea water was measured. One-tenth ml of sea water was removed from each chamber at the end of each run, added to 5 ml of deionized water and 5 ml of Packard Instagel, and assayed in a liquid scintillation counter. An uncorrected systematic error for the light experiments of about one percent was introduced by assaying the sea water on the day following the experiment rather than at the same time that the tips were counted. In all experiments a background correction factor of 11.5 ct min^{-1} was subtracted for each sample counted.

Terminal segment size

To determine the effect of tip size on rate of Ca-45 incorporation, a size sequence of nine tips was incubated for two hours at 0.04% P_{CO_2} and 13 mW cm^{-2} illumination, and then radioassayed. The relative surface areas were computed by tracing microprojector enlargements of the tips onto index cards, cutting the enlargements out, and weighing them.

Although calcification increased as total surface area increased ($r = 0.94$, $p < 0.01$) considerable variability existed (see also Thomas, 1976). Per unit surface area, smaller segments were considerably more

active than were larger ones. Such a growfh gradient (Fig. 5) for terminal segment development is similar to that found for successively younger branch segments (Pearse, 1972), and occurs also in Halimeda (Borowitzka and Larkum, 1976a), and in the frond of Padina (Ikemori, 1970).

RESULTS

Calcification and the CO_2 concentration

Over a nine month period ten P_{CO_2} experiments (Table 5, Fig. 6) were conducted in which six plants were each divided into six parts for simultaneous treatment with six different concentrations of CO_2. Over the P_{CO_2} range 5.5% to 0.04% the resultant pH ranged from 6.5 to 8.7 depending on the particular P_{CO_2} and its flow rate. Optimum calcification occurred using P_{CO_2} levels between 1 and 0.1% (between pH of 7 and 8.3). Plants incubated at optimal P_{CO_2} levels (three to thirty times that of the present atmosphere) calcified about one-third faster than those treated with normal air (P_{CO_2} = 0.04%).

Calcification was correlated positively with the calculated concentration of HCO_3^- expressed as percent of total carbon ($r = 0.86$, $p < 0.01$) (Table 6) (see also Riley and Skirrow, 1966, 1975; Whitfield, 1974).

The killed controls calcified at much lower rates than the living plants (Fig. 6).

Increasing $NaHCO_3$ and Na_2CO_3 concentrations

Experiment B-1: This experiment (Table 7, Fig. 7) tested the effect upon calcification of increasing the $NaHCO_3$ concentration. Increments of $NaHCO_3$ (1 to 16 mM) were added and air (P_{CO_2} = 0.23%) was bubbled through the chambers. The pH ranged from 7.8 (no added $NaHCO_3$) to 8.1 (16 mM added). Calcification increased in living plants almost logarithmically as $NaHCO_3$ increased. Between 1 and 8 mM $NaHCO_3$, the killed controls remained at exchange levels. Addition of 16 mM $NaHCO_3$ resulted in Ca-45 incorporation in the killed controls similar to that in the living plants.

Experiment B-2: This experiment was similar to experiment B-1 except that P_{CO_2} = 0.04% and the pH decreased from 8.6 (no added $NaHCO_3$) to 8.3 (16 mM $NaHCO_3$ added). Calcification in living plants increased nearly linearly as a function of increasing $NaHCO_3$ concentration (Fig. 7). The killed controls (1 to 4 mM added $NaHCO_3$) also showed only a slight increase whereas addition of 8 and 16 mM $NaHCO_3$ to the killed controls caused a marked increase in Ca-45 uptake that surpassed that of living plants.

Experiment C-1: This experiment differed from the previous two in that Na_2CO_3 was used instead of $NaHCO_3$. The addition of Na_2CO_3 (1 to 6 mM) increased pH levels beyond those obtained by bubbling air (P_{CO_2} = 0.04%) alone through the sea water. The resultant pH ranged from 8.5 (no added Na_2CO_3) to 9.4 (6 mM added). In the living plants, calcification showed a nearly direct linear correlation with the Na_2CO_3 concentration. The killed controls increased more rapidly at the higher concentrations (Fig. 7).

Buffers

Experiments H-1 and H-2: My attempts to separate the effect of pH from that of the different carbon species by using buffers are summarized in Table 8. Experiment H-1 simultaneously tested the effects on six different plants of five different P_{CO_2} levels (ranging from 0.04 to 1% P_{CO_2}) on calcification in the presence of 20 mM HEPES buffer. Two-way analysis of variance (Table 9) showed that a difference existed both between P_{CO_2} levels and between plants. Each one of the six plants calcified at a higher rate at P_{CO_2} = 1% than at any of the other levels.

Experiment H-2 simultaneously compared TRIS, HEPES, and $NaHCO_3$ buffers at the P_{CO_2} levels of 0.04 and 0.26%. Two-way analysis of variance (Table 9) showed that a difference in calcification existed at P_{CO_2} = 0.04% between TRIS, HEPES and sea water to which no extra $NaHCO_3$ was added but that the plants weren't different. The same test (Table 9) but with P_{CO_2} = 0.26%, TRIS, HEPES, and sea water buffered with added $NaHCO_3$ again showed the treatments to differ.

The living plants in Experiment H-2 treated with TRIS and HEPES did not incorporate Ca-45 faster than the dead controls. Living controls in plain sea water and a P_{CO_2} of 0.04% incorporated Ca-45 about twice as fast as the dead controls. However, this was less than half the uptake obtained for both living plants and killed controls with a P_{CO_2} of 0.26% and the addition of successive $NaHCO_3$ increments (Table 8) to keep the pH around 8.2.

DISCUSSION

My research has shown that increasing the partial pressure of carbon dioxide in sea water increases calcification in the coralline alga Bossiella orbigniana (Fig. 6). This increase is correlated directly with the HCO_3^- concentration expressed as percent of total carbon. Maximum calcification occurred at concentrations several times those of current atmospheric levels; and concentrations of CO_2 greater than 50 times that of current atmospheric levels were required before calcification fell below that obtained using present atmospheric concentrations. In my experiments pH dropped more than the ½ unit given by Plass (1956). It should be noted that when maximum calcification occurred, $[CO_2] \approx [CO_3^{2-}]$ and $[HCO_3^-]$ was at its maximum (as percent of total carbon). Decrease in calcification from optimum levels closely coincided with increasing CO_2.

During the two hour incubation period, the pH consistently rose 0.1 to 0.3 units in the chambers into which air (P_{CO_2} = 0.04%) was being bubbled. This did not occur at other CO_2 levels. Possible explanations are that the plants (1) utilized CO_2 faster than it was being delivered, (2) removed HCO_3^- or CO_3^{2-} faster than the hydration of CO_2 permitted their formation (Skirrow, 1975), and (3) released a product such as OH^- faster than compensation by CO_2 could occur. This rise in pH suggests that not only would a higher atmospheric concentration of CO_2 be needed to enhance growth rates, but also adequate mixing as well. This is supported by Smith and Kinsey (1976), who report that seaward portions of coral reefs where adequate mixing would be expected produce five times as much calcium carbonate as do the protected areas. They suggest that

the difference is probably largely a function of water motion.

The near linearity at high pH values of Ca-45 uptake by the living plants, even at concentrations that caused marked precipitation in the killed controls (Fig. 7) strongly suggests that living plants are able to control calcification rates. This is in agreement with the work of a number of investigators, as summarized by Clark (1976), who suggest that marine organisms are able to regulate calcification, even under conditions where the solubility product of $CaCO_3$ is exceeded.

According to Chalker (1976) the suggested logarithmic uptake (my Fig. 7) at P_{CO_2} = 0.26% and increasing $NaHCO_3$ concentration can be explained with Michaelis - Menten kinetics. The nearly linear uptake at P_{CO_2} = 0.04% and increasing $NaHCO_3$ or Na_2CO_3 concentrations suggests that simple diffusion may be rate-limiting at higher pH levels.

Since calcification in coralline algae is poorly understood, comparison with other calcifying organisms may be helpful. In coccolithophorids and in corals, membrane transport must be considered because Ca^{++} and carbon species must cross a membrane to reach the calcification site. However, in Halimeda and Bossiella the Ca^{++} or carbon species would not have to pass through a membrane and the cells adjacent to the calcification sites may modify the environment where calcification takes place (see Borowitzka and Larkum, 1976a). Such differences suggest that generalizations comparing calcifying organisms should be proposed with caution.

Somewhat similar results to my P_{CO2} experiments (S-1 to S-10) were reported by Paasche (1964) for the unicellular marine alga Coccolithus huxleyi. This organism calcified most rapidly under his conditions at levels similar to each other at pH = 7.5, 8.0, and 8.5. The relative

calcification level dropped about 50% at pH = 9.1 and 100% at pH = 6.3. He used TRIS buffer, which inhibited calcification in my organisms (Table 8) and may have been responsible for the similarity in his calcification rates from pH = 7.5 to 8.5 in Coccolithus huxleyi; this is the optimum working range for TRIS (Smith and Hood, 1964).

Changing the P_{CO_2} changes the pH and the concentrations of the various carbon species in sea water (Fig. 4). This close relationship between pH and P_{CO_2} makes it difficult to establish cause and effect relationships when dealing with the CO_2 system. Lucas (1974) claims that both CO_2 and HCO_3^- are utilized by Chara, a carbonate precipitating fresh water green alga. Jolliffe and Tregunna (1970) present evidence that HCO_3^- and not CO_2 is utilized by several different marine algae. The rate of coccolith formation in Coccolithus huxleyi reaches a maximum level at about 20 mM total carbon concentration according to Paasche (1964). Natural sea water contains only about 2 mM total carbon. Paasche (1964) and Crenshaw (1964) both claim that at least some coccolithophorids utilize HCO_3^-. However, Steemann Nielsen (1975) states that no experiments showing HCO_3^- utilization by marine plants can be shown except for Coccolithus huxleyi, which he considers a special case.

My results suggest that calcification in Bossiella orbigniana may utilize HCO_3^- and that CO_2 and CO_3^{2-} may compete with it (Table 6 and Fig. 4). The occurrence of maximum calcification at maximum HCO_3^-, expressed as percent of the total carbon concentration, and the decrease in calcification as either CO_2 or CO_3^{2-} increases (thus making HCO_3^- a smaller percent of the total carbon), seems more than coincidental.

However, a suitable experiment remains to be devised to convincingly demonstrate this. The possibility remains that we may be dealing with a pH sensitive system whose optimum coincides with bicarbonate values (Fig. 4).

My experiments in which $NaHCO_3$ and Na_2CO_3 concentrations were increased (Fig. 7) help to sort out the effect of inorganic precipitation (as seen in the killed controls). Calcification in the killed controls appears to be a function of the increasing Na_2CO_3 concentration (compare Fig. 4) at least at low concentrations of added carbon. At the higher added concentrations (8 or 16 mM $NaHCO_3$ and 2 mM or more Na_2CO_3) apparently the solubility product of $CaCO_3$ is greatly exceeded, resulting in precipitation onto the killed but not the living plants.

The effects of pH on calcification in the green alga Halimeda were studied by Borowitzka and Larkum (1976b) using a closed system and varying pH with $NaHCO_3$ and various organic buffers. Although they report calcification as being "fairly constant in the pH range 6.5 to 8.2" (based on three points) the highest point of calcification in this range is at a pH similar to my optimum. Possibly if more points or replicates could be used, calcification in Halimeda would be shown to vary with pH in a manner similar to that of Bossiella.

Using Cricosphaera elongata, a different coccolithophorid than that used by Paasche (1964), Swift and Taylor (1966) measured the division rate as a function of pH. They varied pH by using a combination of two different P_{CO_2}'s (0.03% and 5%) and adding 0.1 to 60 mM $NaHCO_3$. They did not use other buffers. Their pH curve is similar to mine (Fig. 6), with both showing maximum calcification at about pH = 7.8. They

claim that pH and not the concentration of the carbon species is responsible for their results. It should be noted that their system was not in equilibrium with the atmosphere (see my Fig. 4).

If the above objections can be met, the similarity of my pH optimum results to those of Swift and Taylor (1966), Paasche (1964), and Borowitzka and Larkum (1976b), suggest that a common biological process may control both the rate of division and calcification in coccolithophorids and that of calcification in Bossiella, and possibly Halimeda.

CONCLUSIONS

1. Calcification was correlated with size of terminal segment, with the smallest (youngest) tips incorporating the most Ca-45 per unit surface area.

2. Maximum calcification of about one-third above normal occurred when bicarbonate concentration, as percent of total carbon, was at its maximum. This occurred when P_{CO_2} was between 1% and 0.1%, probably between 0.4% and 0.1%. For my synthetic sea water, the resultant pH range was 7.6 to 8.3.

3. Addition of either $NaHCO_3$ or Na_2CO_3 markedly increased calcification. At high pH, calcification increased in living plants as a nearly linear function of concentration; it increased more rapidly in killed controls. This suggests that the diffusion rate may have been the limiting factor and that simple precipitation was not occurring in the living plants, at least not at the rate it appeared to be increasing in the killed controls.

4. Both HEPES and TRIS buffers at 20-25 mM concentration reduced calcification to the level of the killed controls. Only when the experiment using HEPES had continued long enough for the buffer to become saturated (as seen by changing pH) did calcification begin to occur.

5. $NaHCO_3$ functioned well as a buffer and increased calcification, but was not useful for separating the effects of the concentrations of the various carbon species from those of pH since it is itself a carbon species.

6. Calcification is dependent on a number of factors related to the pH-P_{CO_2} system. At high pH (low P_{CO_2}) increasing $[CO_3^{2-}]$ may diffuse through the cell wall and precipitate onto available calcification lattice sites. In the pH range 6.5 to 8.7 the calcification rate was correlated directly with the $[HCO_3^-]$ expressed as percent of total carbon. This suggests that pH and/or HCO_3^- may be rate limiting. Both CO_2 and CO_3^{2-} may compete with the process when the system is in equilibrium. The possibility that molecular CO_2 may be responsible for the increase in calcification up to 0.26% or more P_{CO_2} has not been ruled out, but this does not seem to be the simplest explanation.

TABLE 1. EXPERIMENTAL PARAMETERS (in hours and minutes)

EXPERIMENT	DATE	COLLECTION TIME (PST) (±30 min)	START OF PREINCUBATION	TOTAL PREINCUBATION TIME (min)	BEGIN INCUBATION	TOTAL INCUBATION TIME (min)	RINSE TIME (min)	DRY TIME (min)	BLEACH TIME (hrs)
				Light Experiments					
L-1	4 July 76	10:30	16:30	50	17:20	145	22	155	68
L-2	12 July 76	9:00	13:00	75	14:15	140	5	225	77
				Standard Experiments					
S-1	6 Sept 76	8:55	12:10	70	13:20	130	10	15	74
S-2	8 Oct 76	8:30	12:55	73	14:09	120	8	17	72
S-3	17 Nov 76	9:45	13:00	110	14:54	120	2	3	72
S-4	18 Nov 76	9:45	12:45	58	13:48	120	8	7	89
S-5	27 Dec 76	9:45	12:44	67	13:51	120	9	4	72
S-6	28 Dec 76	9:45	12:50	60	13:50	120	4	7	72
S-7	30 Dec 76	10:07	13:35	60	14:35	120	4	5	72
S-8	11 Feb 77	9:45	14:10	53	15:03	65	4	8	73
S-9	14 Feb 77	9:45	12:05	62	13:07	120	5	5	73
S-10	21 Mar 77	9:40	12:15	60	13:15	120	5	2	72
				Na_2CO_3 Experiment					
C-1	12 Dec 76	9:22	13:00	95	14:35	75	3	10	72
				$NaHCO_3$ Experiments					
B-1	19 Dec 76	9:45	13:20	104	15:04	120	6	16	72
B-2	29 Dec 76	9:52	13:24	86	14:50	80	8	8	75
				HEPES and TRIS Buffer Experiments					
H-1	26 Nov 76	9:30	12:26	55	13:24	60	5	6	75
H-2	23 Jan 77	10:07	13:27	95	14:52	65	4	22	-

TABLE 2. BEGINNING AND ENDING pH VALUES FOR EXPERIMENTS S-1 TO S-10

P_{CO_2} (%)	5.46	2.26	1.05	0.93	0.39	0.26	0.23	0.11	0.04
Exp. S-1	-	6.92-6.97	-	7.25-7.24	7.83-7.68	-	8.01-7.88	8.42-8.49	8.59-8.81
S-2	-	-7.06	-	-7.34	-7.67	-	-7.96	-8.39	-8.99
S-3	-	6.86-6.85	-	7.13-7.10	7.65-7.55	-	7.89-7.78	8.33-8.31	8.67-8.78
S-4	-	-6.85	-	-7.14	-7.60	-	-7.85	-8.17	-8.83
S-5	-	6.77-6.76	7.10-7.04	-	7.54-7.47	-	7.82-7.74	8.24-8.19	8.52-8.61
S-6	-	6.80-6.73	7.10-7.01	-	7.53-7.46	-	7.84-7.74	8.30-8.23	8.53-8.67
S-7	6.29-6.36	6.69-6.69	7.01-6.99	-	7.46-7.45	-	7.74-7.74	-	8.42-8.73
S-8	6.47-6.50	6.82-6.80	7.13-7.11	-	-	7.83-7.81	-	8.51-8.41	8.59-8.71
S-9	6.58-6.58	6.88-6.88	7.14-7.14	-	-	7.80-7.80	-	8.42-8.41	8.64-8.82
S-10	6.62-6.65	6.87-6.89	7.15-7.14	-	-	7.82-7.78	-	8.22-8.19	8.62-8.69
n	4	10	6	4	7	3	7	9	10
$\bar{x}$ ± S.E.	6.51±0.07	6.83±0.03	7.09±0.02	7.21±0.05	7.59±0.04	7.81±0.01	7.84±0.09	8.32±0.04	8.70±0.04
Combined for Fig. 6 →			7.14±0.03			7.83±0.04			

TABLE 3. PARAMETERS USED FOR CALCULATION OF CARBON SPECIES CONCENTRATIONS FOR 19°C AND 19‰ Cl SEA WATER[1]

Solubility of CO_2 in sea water (α_s)	340×10^{-4} moles l^{-1} atm^{-1}
K'_{1l} = first apparent dissociation constant of carbonic acid	9.419×10^{-7} moles l^{-1}
$pK'_{1l} = -\log [K'_{1l}]$	6.026
K'_{2l} = second apparent dissociation constant of carbonic acid	6.577×10^{-10} moles l^{-1}
$pK'_{2l} = -\log [K'_{2l}]$	9.182

Partial pressure of carbon dioxide 0.04% to 5.46% = 400 to 54,600 ppm = .0004 atm to 546 atm

$[H_2CO_3] = P_{CO_2} \cdot \alpha_s$ where H_2CO_3 includes molecular CO_2 concentration

$[HCO_3^-] = P_{CO_2} \cdot \alpha_s \cdot K'_{1l} / [a_{H^+}]$

$[CO_3^{2-}] = P_{CO_2} \cdot \alpha_s \cdot K'_{1l} \cdot K'_{2l} / [a_{H^+}]^2$

Total carbon = $[H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]$

[1]From Skirrow (1975) and Park (1969).

TABLE 4. LIGHT INTENSITY AND CALCIFICATION[1]

Light intensity (mW cm^{-2})	0	0.1	0.4	0.8	1.0	1.2	1.3	2.3	5.0 ↓	10
Experiment L-1[2]	62±4	77±4	82±5				75±6	83±5	89±5	
Experiment L-2	84±7			93±11	86±7	90±8			91±7	73±8

[1]Each entry is the mean ±1 S.E. of 6 plants, given in ct min^{-1} tip^{-1} hr^{-1}. See Table 9 for ANOVA.
[2]Experiment L-1 was held overnight in the dark (30 hrs) before use, whereas Experiment L-2 was run on the same day that the plants were collected.
↓All experiments except L-1 and L-2 were run at this light intensity.

TABLE 5. CALCIFICATION IN EXPERIMENTS S-1 TO S-10

EXPERIMENT	PARTIAL PRESSURE OF CO_2 (% in air) 5.45	2.26	1.05	0.93	0.39	0.26	0.23	0.11	0.04
				Living plants[1]					
S-1	-	42±6	-	56±7	68±4	-	60±6	55±6	35±4
S-2	-	65±4	-	73±7	83±2	-	79±5	84±4	61±8
S-3	-	61±4	-	58±6	64±8	-	75±4	77±8	58±7
S-4	-	55±4	-	75±5	93±7	-	99±7	75±7	68±3
S-5	-	58±4	67±6	-	67±7	-	87±10	74±6	68±6
S-6	-	50±6	55±8	-	66±8	-	48±6	61±11	41±7
S-7	26±4	53±6	59±9	-	58±7	-	58±5	-	48±2
S-8	41±4	64±6	64±6	-	-	81 5	-	67±3	59±6
S-9	43±4	70±6	83±3	-	-	92 5	-	83±6	68±4
S-10	49±5	72±5	83±10	-	-	106 15	-	102±10	79±8
n	4	10	6	4	7	3	7	9	10
$\bar{\bar{x}}$±1 S.E.	40±5	59±3	69±5	66±5	71±5	93	72±7	75±5	58±5
			n 10			10			
Averaged for Figure 6			$\bar{\bar{x}}$ ±1 S.E. 67±4			79±6			
				Killed Controls[2]					
S-5	-	10	12	-	8	15	-	14	9
S-6	-	17	16	-	9	9	-	13	12
S-7	18	11	9	-	7	11	-	-	9
S-8	42	21	76	-	-	19	-	28	38
S-9	17	23	24	-	-	17	-	14	20
S-10	27	31	26	-	-	23	-	25	29
n	4	6	6	-	3	6	-	5	6
$\bar{\bar{x}}$ ±1 S.E.	26±6	18±4	21±5		8±1	19±5		19±3	19±8

[1]Each entry is the mean ±1 S.E. of 6 plants expressed as ct min^{-1} tip^{-1} hr^{-1}. Each plant was represented by 6 tips counted simultaneously. $\bar{\bar{x}}$ is the mean of the means. See Table 9 for ANOVA.

[2]Each entry represents 6 tips from one plant counted simultaneously and expressed as ct min^{-1} tip^{-1} hr^{-1}.

TABLE 6. CORRELATION COEFFICIENTS COMPARING CALCIFICATION WITH HCO_3^- EXPRESSED AS PERCENT OF TOTAL CARBON

PARTIAL PRESSURE CO_2 (%)	5.45	2.26	1.05	0.93	0.39	0.26	0.23	0.11	0.04	r	p
HCO_3^- (% of total carbon)[1]	70	83	91	91	45	95	95	94	88	0.87	< 0.01
Calcification[2]	40	59	69	66	71	93	72	75	58		

[1] From Figure 4.
[2] From Table 5 and Figure 6 (ct min^{-1} tip^{-1} hr^{-1}).

TABLE 7. $NaHCO_3$ AND Na_2CO_3 EXPERIMENTS

EXPERIMENT B-1 $NaHCO_3$ ADDED. P_{CO_2} = 0.23%						
Amount added (mM)	0	1	2	4	8	16
Beginning pH	7.84	7.82	7.83	7.85	7.84	7.83
Ending pH	7.74	7.83	7.88	8.10	8.15	8.28
Calcification (ct min^{-1} tip^{-1} hr^{-1})						
Living plants $\bar{x}$ ±1 S.E. (n=6)	84±5	86±5	96±11	119±7	126±9	141±14
Killed controls (n=1)	7	9	11	11	12	115

EXPERIMENT B-2. $NaHCO_3$ ADDED. P_{CO_2} = 0.04%						
Amount added (mM)	0	2	3	4	8	16
Beginning pH	8.57	8.33	8.27	8.26	8.21	8.14
Ending pH	8.66	8.58	8.52	8.54	8.43	8.40
Calcification (ct min^{-1} tip^{-1} hr^{-1})						
Living plants $\bar{x}$ ±1 S.E. (n=6)	58±5	90±4	95±9	115±13	149±17	218±12
Killed controls (n=1)	23	36	38	42	92	263

EXPERIMENT C-1. Na_2CO_3 ADDED. P_{CO_2} = 0.04%						
Amount added (mM)	0	1	2	3	4	6
Beginning pH	8.53	8.97	9.19	9.33	9.45	9.56
Ending pH	8.53	8.87	9.03	9.17	9.34	9.33
Calcification (ct min^{-1} tip^{-1} hr^{-1})						
Living plants $\bar{x}$ ±1 S.E. (n=6)	42±3	70±14	89±7	141±12	173±13	247±24
Killed controls (n=1)	15	26	86	224	301	443

TABLE 8. EFFECT OF BUFFERS ON CALCIFICATION

P_{CO_2} (% in air)	BUFFER	BUFFER CONC(mM)	INITIAL pH	FINAL pH	LIVING PLANTS[1]	KILLED CONTROLS[2]
			Experiment H-1			
0.04	HEPES	20	7.70	7.79	29±4	none
0.11	HEPES	20	7.68	7.75	28±4	none
0.23	HEPES	20	7.67	7.67	30±3	none
0.39	HEPES	20	7.66	7.63	32±6	none
1.05	HEPES	20	7.58	7.34	51±7	none
			Experiment H-2			
0.04	TRIS	25	8.15	8.19	35±3	32
0.26	TRIS	25	8.08	8.10	39±4	39
0.04	HEPES	25	8.11	8.12	43±6	44
0.26	HEPES	25	8.10	8.11	46±5	45
0.04	Control		8.48	8.65	85±6	45
0.26	$NaHCO_3$	26[3]	8.15	8.19	182±12	92

[1]Average of 6 plants ±1 S.E. expressed as ct min^{-1} tip^{-1} hr^{-1}. See Table 9 for ANOVA.
[2]One plant expressed as ct min^{-1} tip^{-1} hr^{-1}.
[3]Added in successive millimolar increments of 8, 2, 4, 4, 4, and 4 to keep pH stable.

TABLE 9. ANALYSES OF VARIANCE OF DATA (Via General Linear Hypothesis or Two-way ANOVA)

SOURCE	D.F.	MEAN SQUARE	F	D.F.	MEAN SQUARE	F	D.F.	MEAN SQUARE	F
	Experiment L-1			Experiment L-2			Experiment C-1		
Treatment	5	1746	5.03**	5	1286	1.59	5	114718	43.46***
Plant	5	1119	3.23*	5	4540	5.60**	4	9703	3.68**
Error	25	347		25	810		20	2640	
	Experiment B-1			Experiment B-2			Experiment H-1		
Treatment	5	14330	5.37**	5	75480	39.66***	4	2139	5.81**
Plant	5	2318	0.87	5	7272	3.82**	5	1561	4.24**
Error	25	2667		25	1903		20	368	
	Experiment H-2 (P_{CO_2} = 0.26%)			Experiment H-2 (P_{CO_2} = 0.04%)			Experiments S-1 to S-10		
Treatment	2	162035	111.9***	2	17001	29.11**	6	28339	41.58***
Plant	5	1314	0.91	5	336	0.57	50	2512	3.68***
Experiment	-	-	-	-	-	-	9	19464	28.56***
Error	10	1448		10	584		294	681	

Probability of a larger F: * <0.05, ** <0.01, *** <0.001

Figure 1. Plexiglass chamber with plants in sea water.

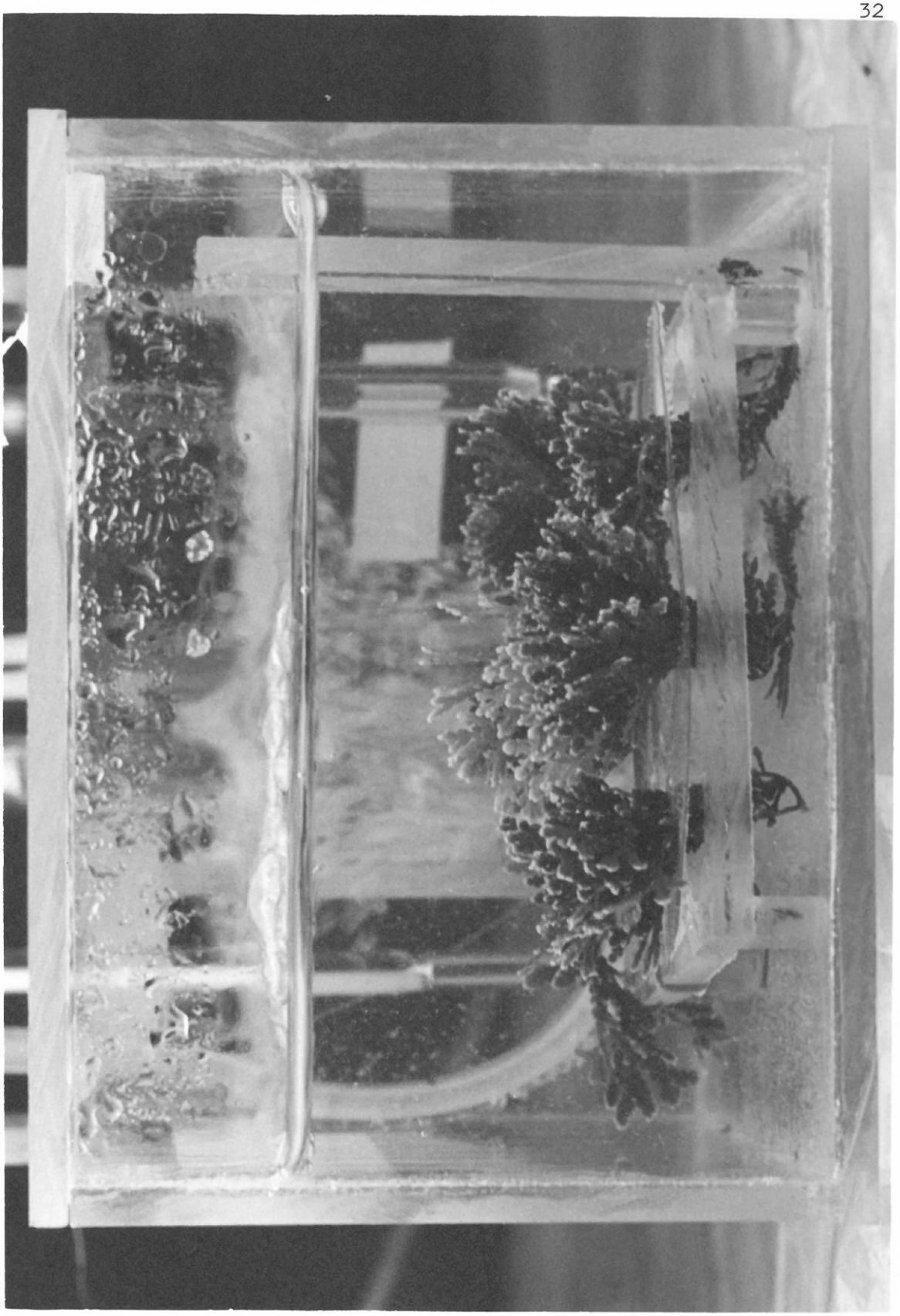


Figure 2. General view of experimental apparatus.

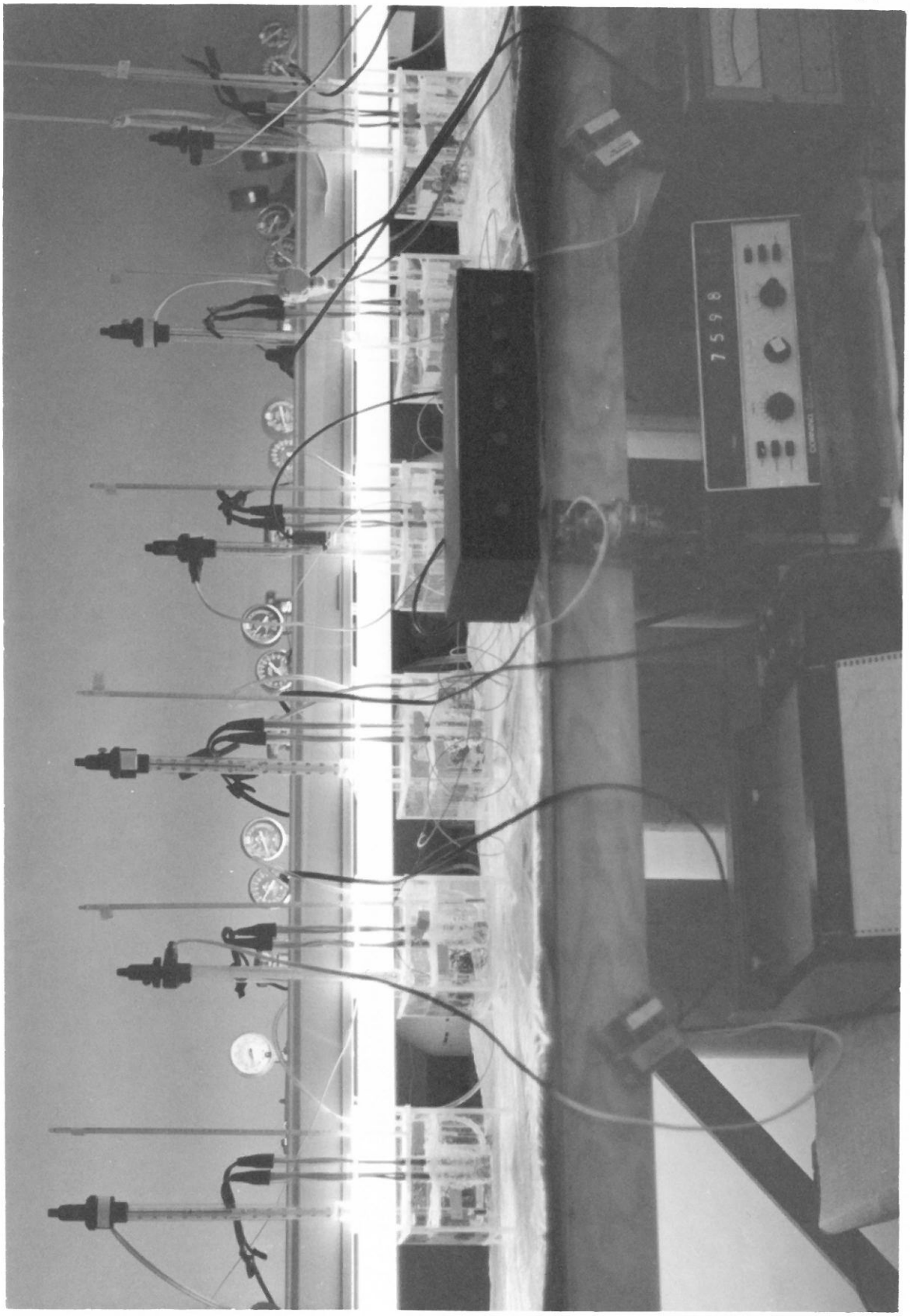

7598

Figure 3. Relation between partial pressure of carbon dioxide in air (P_{CO_2}) and pH. The equation for the least squares line is pH = 1.065 ($-\log P_{CO_2}$) + 5.07. The points are based on the sea water pH readings obtained in Experiments S-1 to S-10 and the measured P_{CO_2} values of the air in the tanks.

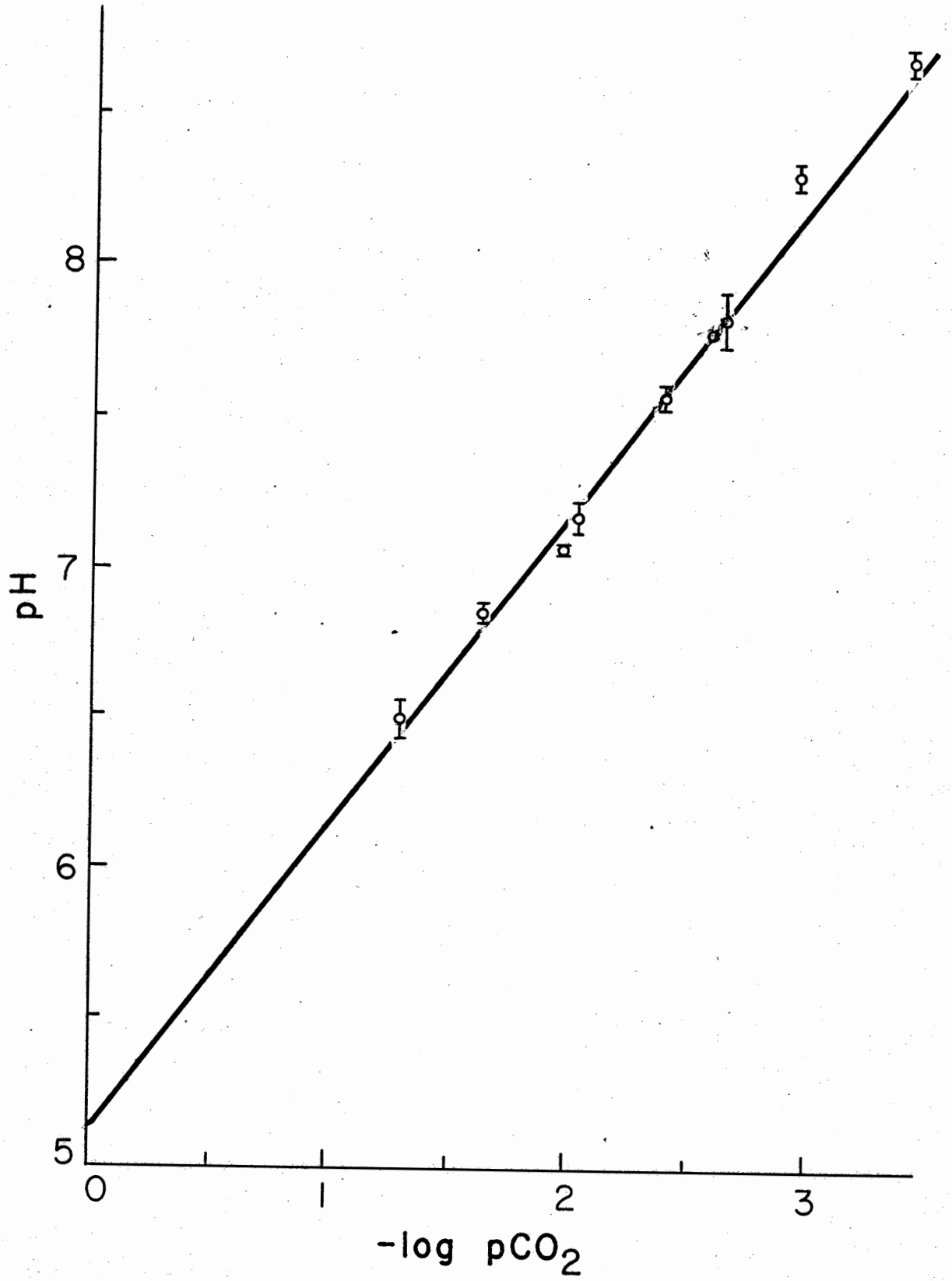

pH
8
7
6
5
0
1
2
3
-log pCO2

Figure 4. Concentrations of carbon species, expressed as percent of total carbon, in synthetic sea water, calculated from the tank P_{CO_2} values and the resultant sea water pH values. See Table 3 for parameters.

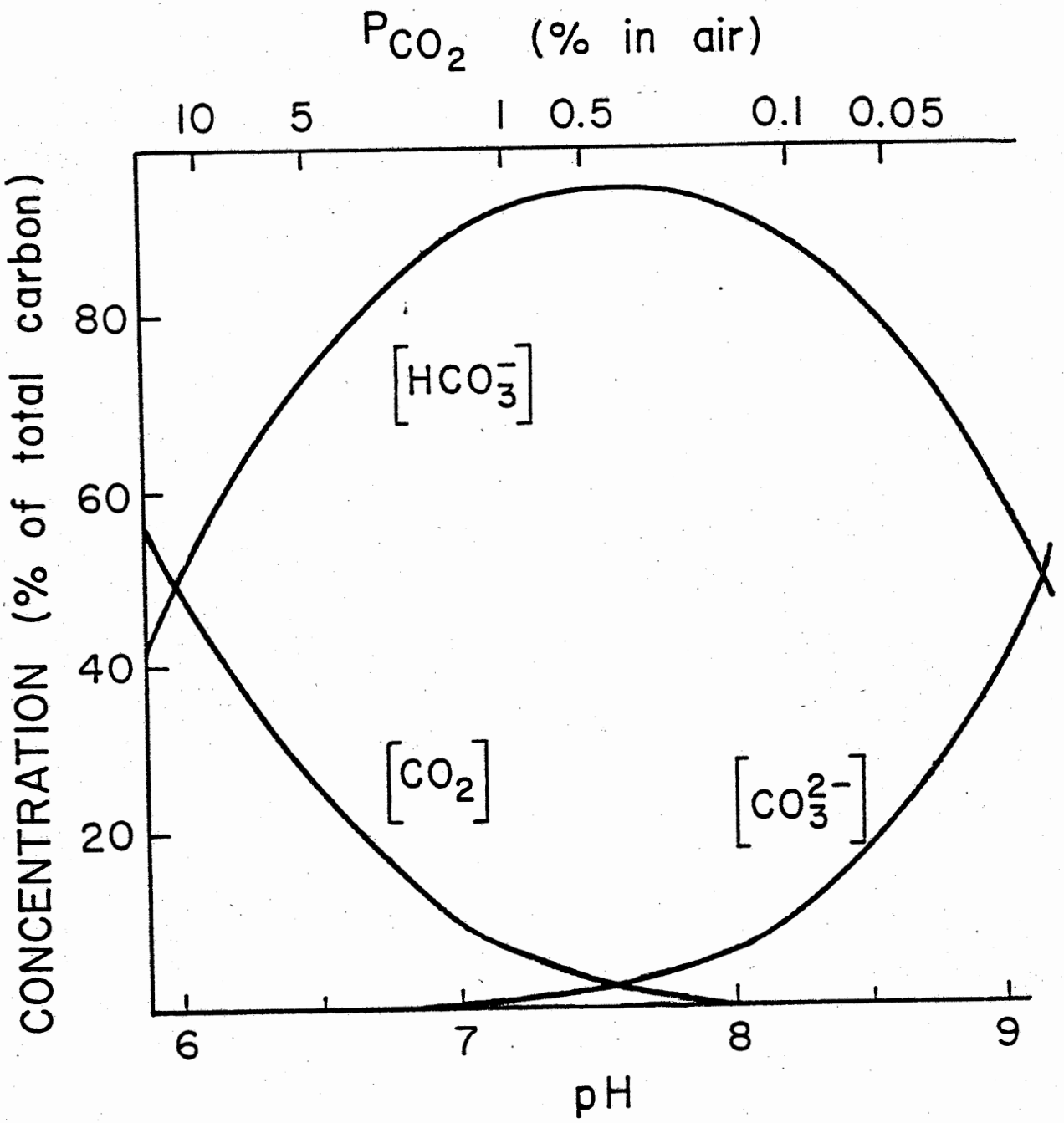

P_{CO_2} (% in air)
10
5
1
0.5
0.1
0.05
CONCENTRATION (% of total carbon)
80
60
40
20
$[HCO_3^-]$
$[CO_2]$
$[CO_3^{2-}]$
6
7
8
9
pH

Figure 5. Calcification as a function of area of terminal segment. Each point represents one segment. The units of total surface area are relative only and refer to the weights of the paper used to estimate area.

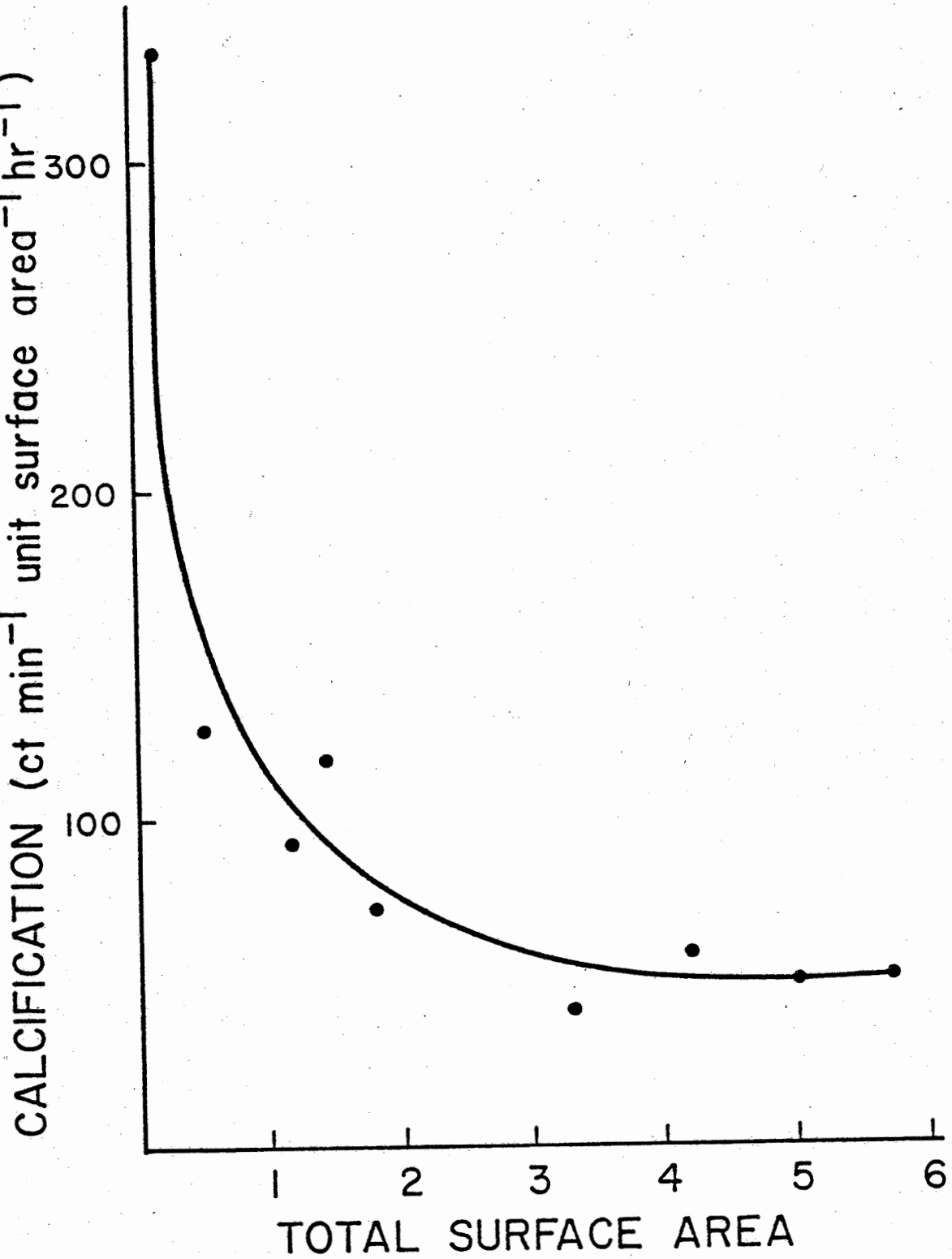

CALCIFICATION (ct min⁻¹ unit surface area⁻¹ hr⁻¹)
300
200
100
1
2
3
4
5
6
TOTAL SURFACE AREA

Figure 6. Calcification of Bossiella orbigniana expressed as a function of measured pH (lower scale) and of calculated P_{CO_2} (top scale). Note that the top scale is logarithmic and is derived from the least squares line of Figure 3. Each open circle represents the mean ±1 S.E. of (n) experiments of 6 living plants each. Each closed circle represents the mean ±1 S.E. of (n) experiments of 1 killed control plant each; note that the low point of pH = 7.6 is based on a smaller sample. In all cases each plant is represented by the average of 6 tips assayed simultaneously. Based on data from Table 5. See Table 9 for ANOVA.

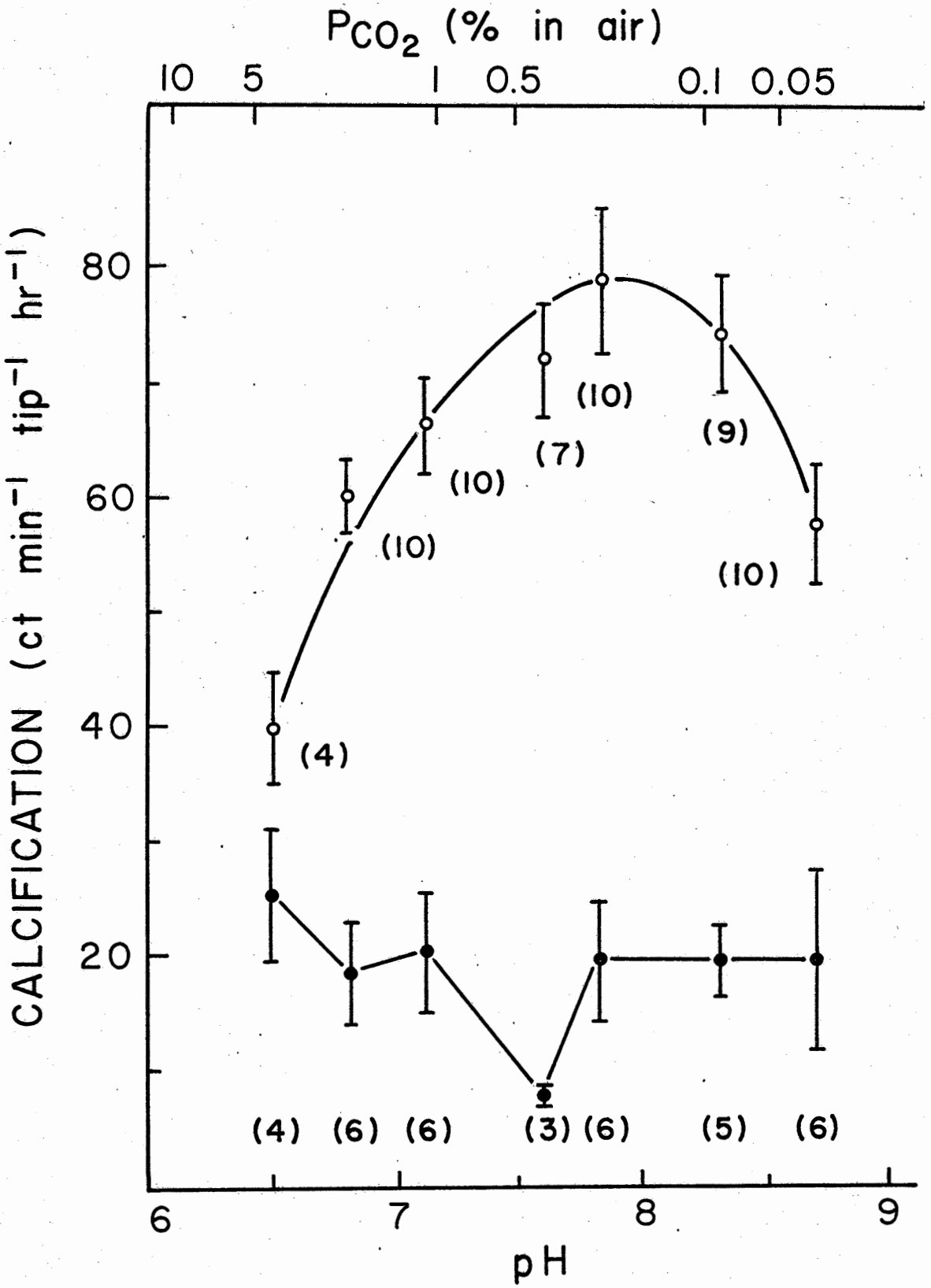
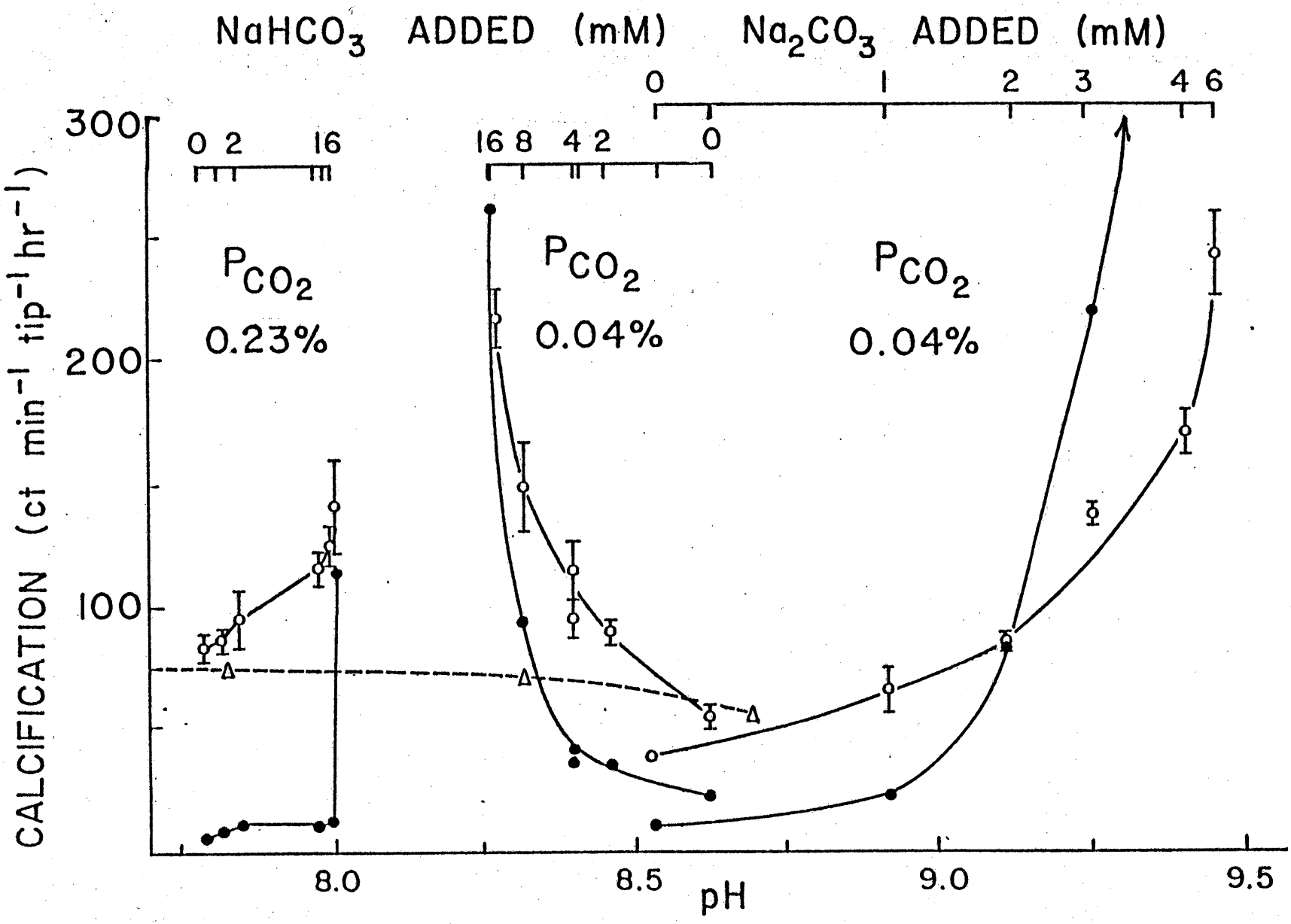

P_{CO_2} (% in air)
10
5
1
0.5
0.1
0.05
CALCIFICATION (ct min^{-1} tip^{-1} hr^{-1})
80
60
40
20
(4)
(10)
(10)
(7)
(10)
(9)
(10)
(4)
(6)
(6)
(3)
(6)
(5)
(6)
6
7
8
9
pH

Figure 7. Composite of two experiments (P_{CO_2} = 0.23% and 0.04%) in which $NaHCO_3$ was added and one (P_{CO_2} = 0.04%) in which Na_2CO_3 was added to the sea water. Amounts added (millimolar increments) are given at the top of the figure. Open circles (-o-): mean ±1 S.E. of living plants. Closed circles (-●-): 1 killed control plant. Each plant is represented by 6 tips assayed simultaneously. Triangles (- - Δ - -): the portion of the standard-experiments curve (Fig. 6) that falls in this pH range. Experiment B-1 (at far left) was incubated for 2 hrs, B-2 (in center) for 1.3 hrs, and C-1 (at far right) for 1.2 hrs. Each has been converted for comparative purposes to 1 hr, assuming linearity over time (see Pearse, 1972). Note that each point in this figure is based on only one experiment, in contrast to those in Fig. 6.


NaHCO3 ADDED (mM)
Na2CO3 ADDED (mM)
0 2 16
16 8 4 2 0
0 1 2 3 4 6
PCO2 0.23%
PCO2 0.04%
PCO2 0.04%
CALCIFICATION (ct min-1 tip-1 hr-1)
300
200
100
8.0
8.5
9.0
9.5
pH

LITERATURE CITED

Adams, J.A.S., M.S.M. Mantovani, and L.L. Lundell. 1977. Wood versus fossil fuel as a source of excess carbon dioxide in the atmosphere: a preliminary report. Science 196:54-56.

Bolin, B. 1977. Changes of land biota and their importance for the carbon cycle. Science 196:613-615.

Borowitzka, M.A. and A.W.D. Larkum. 1976a. Calcification in the green alga Halimeda. II. The exchange of Ca^{2+} and the occurrence of age gradients in calcification and photosynthesis. Jour. Exp. Bot. 27:864-878.

Borowitzka, M.A. and A.W.D. Larkum. 1976b. Calcification in the green alga Halimeda. III. The sources of inorganic carbon for photosynthesis and calcification and a model of the mechanism of calcification. Jour. Exp. Bot. 27:879-893.

Borowitzka, M.A. and A.W.D. Larkum. 1976c. Calcification in the green alga Halimeda. IV. The action of metabolic inhibitors on photosynthesis and calcification. Jour. Exp. Bot. 27:894-907.

Borowitzka, M.A. and A.W.D. Larkum. 1977a. Calcification in the green alga Halimeda. I. An ultrastructure study of thallus development. Jour. Phycol. 13:6-16.

Borowitzka, M.A. and A.W.D. Larkum. 1977b. Algal calcification. To be published in: Oceanology and Marine Biology: Annual Review, Vol. 15.

Chalker, B.E. 1976. Calcium transport during skeletogenesis in hermatypic corals. Comp. Biochem. Physiol. 54A:455-459.

Clark, G.R., II. 1976. Shell growth in the marine environment: approaches to the problem of marginal calcification. Amer. Zool. 16:617-626.

Clausen, C.D. 1972. Factors affecting calcification processes in the hermatypic corals Pocillopora damicornis and Porites compressa. Ph.D. Dissertation. Loma Linda Univ. 95 pp.

Coyne, L. 1975. Chemistry in natural water systems. Jour. of Chem. Ed. 52:796-800.

Crenshaw, M.A. 1964. Coccolith formation by two marine coccolithophorids, Coccolithus huxleyi and Hymenomonas sp. Ph.D. Dissertation. Duke Univ. 82 pp.

Darley, W.M. 1974. Silification and calcification. Botanical Monographs No. 10. In W.D.P. Stewart (ed.). Algal Physiology and Biochemistry, pp. 655-675. University of California Press, Berkeley and Los Angeles.

Darwin, C.R. 1842. The structure and distribution of coral reefs. London: Smith, Elder and Co. 214 pp.

Garrels, R.M., A. Lerman, and F.T. Mackenzie. 1976. Controls of atmospheric O_2 and CO_2: past, present, and future. Amer. Sci. 64: 306-315.

Goreau, T.F. 1959. The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. Biol. Bull. 116:59-75.

Goreau, T.F. 1963. Calcium carbonate deposition by coralline algae and corals in relation to their roles as reef-builders. Ann. N.Y. Acad. Sci. 109:127-167.

Harvey, H.W. 1957. The chemistry and fertility of sea waters. Cambridge University Press, Cambridge. 234 pp.

Ikemori, M. 1970. Relation of calcium uptake to photosynthetic activity as a factor controlling calcification in marine algae. Bot. Mag. Tokyo 83:152-162.

Jolliffe, E.A. and E.B. Tregunna. 1970. Studies on HCO_3^- ion uptake during photosynthesis in benthic marine algae. Phycol. 9:293-303.

Kolesar, P.T. 1973. Factors affecting the magnesium content of calcite secreted by some articulated coralline algae. Ph.D. Dissertation. University of California, Riverside. 131 pp.

Lucas, W.J. 1974. Photosynthetic fixation of ^{14}Carbon by internodal cells of Chara corallina. Jour. Exp. Bot. 26:331-346.

Menard, H.W. 1964. Marine geology of the Pacific. McGraw-Hill Book Co., New York. 271 pp.

Moss, D.N. 1976. Studies on increasing photosynthesis in crop plants. In R.H. Burris and C.C. Black (eds.). CO_2 Metabolism and Plant Productivity, pp. 31-41. University Park Press, Baltimore.

Paasche, E. 1964. A tracer study of the inorganic carbon uptake during coccolith formation and photosynthesis in the coccolithophorid Coccolithus huxleyi. Physiologia Pl. Suppl. 3:1-82.

Paasche, E. 1965. The effect of 3-(p-chlorophenyl)-1, 1-dimethylurea (CMU) on photosynthesis and light-dependent coccolith formation in Coccolithus huxleyi. Physiologia Pl. 18:138-145.

Paasche, E. 1968. Biology and physiology of coccolithophorids. Ann. Rev. Microbiol. 22:71-86.

Park, P.K. 1969. Oceanic CO_2 system: an evaluation of ten methods of investigation. Limnol. Oceanogr. 14(2):179-186.

Pearse, V.B. 1972. Radioisotopic study of calcification in the articulated coralline alga Bossiella orbigniana. Jour. Phycol. 8: 88-97.

Plass, G.N. 1956. Carbon dioxide and the climate. Amer. Sci. 44:302-316.

Pytkowicz, R.M., S.E. Ingle, and C. Mehrbach. 1974. Invariance of apparent equilibrium constants with pH. Limnol. Oceanogr. 19: 665-669.

Riley, J.P. and G. Skirrow (eds.). 1966. Chemical oceanography. Vols. I and II. Academic Press, London. 710 and 508 pp.

Riley, J.P. and G. Skirrow (eds.). 1975. Chemical oceanography. Vols. I and II. 2nd ed. Academic Press, London. 606 and 648 pp.

Roth, A.A. 1974. Factors affecting light as an agent for carbonate production by coral. Geol. Soc. Amer. Abstr. 6(7):932.

Silva, P.C. 1957. Notes on Pacific marine algae. Madrono 14:41-51.

Skirrow, G. 1975. The dissolved gases — carbon dioxide. In J.P. Riley and G. Skirrow (eds.). Chemical Oceanography. 2nd ed. Vol. II, pp. 1-192. Academic Press, London.

Smith, S.V. 1973. Carbon dioxide dynamics: a record of organic carbon production, respiration, and calcification in the Eniwetok Reef flat community. Limnol. Oceanogr. 18(1):106-120.

Smith, S.V. and D.W. Kinsey. 1976. Calcium carbonate production, coral reef growth, and sea level change. Science 194:937-939.

Smith, W.H., Jr. and D.W. Hood. 1964. pH measurement in the ocean: a sea water secondary buffer system. In Y. Miyake and T. Koyama (eds.). Ken Sugawara Festival Volume, Recent Researches in the Fields of the Hydrosphere, Atmosphere and Nuclear Geochemistry, pp. 185-202. Maruzen Co., Tokyo.

Stark. L.M., L. Almodovar, and R.W. Krauss. 1969. Factors affecting the rate of calcification in Halimeda opuntia (L.) Lamouroux and Halimeda discoidea Decaisne. Jour. Phycol. 5:305-312.

Steemann Nielsen, E. 1975. Marine photosynthesis. American Elsevier Publ. Co., New York. 142 pp.

Stoddart, D.R. 1969. Ecology and morphology of recent coral reefs. Biol. Rev. 44:433-498.

Suess, H.E. 1973. Natural radiocarbon. Endeavor 32:34-38.

Swift, E. and W.R. Taylor. 1966. The effect of pH on the division rate of the coccolithophorid Cricosphaera elongata. Jour. Phycol. 2: 121-125.

Thomas, C.M. 1976. Effects of temperature on calcium uptake in Bossiella orbigniana orbigniana. M.A. Thesis. Loma Linda Univ. 101 pp.

Vandermeulen, J.H., N.D. Davis, and L. Muscatine. 1972. The effect of inhibitors of photosynthesis on zooxanthellae in corals and other marine invertebrates. Mar. Biol. 16:185-191.

Whitfield, M. 1974. The ion-association model and the buffer capacity of the carbon dioxide system in seawater at 25°C and 1 atmosphere total pressure. Limnol. Oceanogr. 19:235-248.

Wilbur, K.M., L.H. Colinvaux, and N. Watabe. 1969. Electron microscope study of calcification in the alga Halimeda (Order Siphonales). Phycol. 8:27-35.

APPENDIX

The appendix contains the data from all 17 experiments. The first six rows represent the plants; the columns represent the treatments. Row 7 contains the treatment means.

The figures were derived using the equation:

$$\text{Calcification } (ct\ min^{-1}\ tip^{-1}\ 2hr^{-1}) = \frac{10{,}000 \text{ counts} \times \text{chamber corr.}}{6 \text{ tips/planchet} \times \text{Exp. Corr.} \times \text{Time (min)}}$$

The chamber correction allowed for differences among treatments due to errors in addition of the isotope to each chamber. The experimental correction compensated for variation in incubation time, time between when experiment was run and the tips counted, and for Ca-45 decay of the $^{45}CaCl_2$ with time. Background counts = 11.5 were subtracted. Six tips were counted simultaneously but each plant part was counted separately. See text for additional information. Note that the appendix figures are for 2 hrs. whereas the text data are for 1 hr.

JULY 4, 1976, Experiment L-1
CHAMBER CORRECTION: 1.055 1.057 1.03 1.007 .989 .883
EXPERIMENT CORRECTION: 0.98970

0 mW cm^{-2}	0.1 mW cm^{-2}	0.4 mW cm^{-2}	1.3 mW cm^{-2}	2.3 mW cm^{-2}	5 mW cm^{-2}
96.30	147.28	160.40	130.17	153.23	132.58
134.48	139.98	152.28	168.33	168.74	119.16
126.54	138.20	154.00	107.00	149.72	107.55
136.06	164.21	144.48	157.74	139.77	126.18
145.30	185.83	161.60	145.51	190.37	160.40
101.30	143.28	206.13	186.14	193.35	143.39
123.33	153.13	163.15	149.15	165.86	131.54

JULY 12, 1976, Experiment L-2
CHAMBER CORRECTION: 1.037 1.011 1.007 .999 .988 .961
EXPERIMENT CORRECTION: 0.98117

0 mW cm^{-2}	0.8 mW cm^{-2}	1.0 mW cm^{-2}	1.2 mW cm^{-2}	5 mW cm^{-2}	10 mW cm^{-2}
178.11	196.91	164.30	197.74	181.18	79.95
171.98	262.83	212.10	225.17	223.22	146.52
141.40	139.14	198.64	155.03	135.45	137.57
127.74	120.80	123.50	153.73	148.03	133.73
161.70	196.91	154.89	134.03	190.45	185.65
219.97	199.47	170.27	205.77	207.59	187.08
166.82	186.01	170.61	178.58	180.98	145.08

SEPTEMBER 6, 1976, Experiment S-1
CHAMBER CORRECTION: .965 .992 .994 1 .956 .971
EXPERIMENT CORRECTION: 1.06060

0.04%	0.11%	0.23%	0.39%	0.93%	2.26%
35.11	104.40	89.66	118.58	80.21	30.47
90.61	111.14	108.28	136.05	94.89	95.72
83.81	90.85	115.38	140.91	131.98	88.95
71.00	130.34	151.37	111.07	139.78	90.15
68.56	68.81	98.19	145.95	75.18	75.44
66.66	148.54	156.09	161.37	151.26	122.81
69.29	109.01	119.83	135.65	112.22	83.92

OCTOBER 8, 1976, Experiment S-2
CHAMBER CORRECTION: 1.003 1.003 .991 1.005 1 .991
EXPERIMENT CORRECTION: 0.81900

0.04%	0.11%	0.23%	0.39%	0.93%	2.26%
155.12	156.35	113.21	154.36	168.42	133.58
85.00	198.27	170.01	150.94	182.67	136.02
108.63	164.30	161.30	162.55	110.94	102.75
114.88	173.71	172.16	170.45	174.51	149.57
183.44	151.78	182.59	174.25	126.09	149.70
83.43	165.83	142.21	175.27	107.50	109.47
121.75	168.37	156.91	164.64	145.02	130.18

NOVEMBER 17, 1976, Experiment S-3
CHAMBER CORRECTION: 1.045 .986 .982 .994 .996 .996
EXPERIMENT CORRECTION: 0.82900

0.04%	0.11%	0.23%	0.39%	0.93%	2.26%
104.50	140.05	139.20	111.40	87.23	127.26
76.92	152.87	173.70	158.86	126.02	125.83
166.69	100.49	163.21	78.92	137.59	123.52
130.45	173.93	140.95	134.90	159.35	115.87
80.68	142.88	118.47	98.42	104.98	85.61
127.91	213.50	163.36	180.65	83.31	147.29
114.52	153.95	149.81	127.19	116.41	120.90

NOVEMBER 18, 1976, Experiment S-4
CHAMBER CORRECTION: .987 .994 .972 1.044 1.012 .99
EXPERIMENT CORRECTION: 1.02400

0.04%	0.11%	0.23%	0.39%	0.93%	2.26%
119.85	134.25	139.60	185.16	155.04	106.80
130.66	108.78	207.31	155.74	141.29	111.22
144.01	189.22	176.16	234.40	189.61	114.48
125.45	172.13	223.57	188.40	117.68	102.13
153.77	164.92	218.44	204.96	144.62	136.32
142.81	127.37	217.77	143.25	150.30	85.33
136.09	149.44	197.14	185.32	149.76	109.38

NOV.27, 1976, Experiment H-1
CHAMBER CORRECTION: 1.059 .997 .977 .979 .99 .997
EXPERIMENT CORRECTION: 0.81629

0.04%	0.11%	0.23%	0.39%	1.05%
63.99	46.29	77.87	77.93	100.85
35.66	48.26	65.96	38.06	149.56
89.96	74.21	60.41	107.90	128.38
46.51	37.86	34.93	40.95	50.84
55.15	41.29	54.61	35.64	80.12
57.36	84.65	65.57	83.09	95.78
58.10	55.43	59.89	63.93	100.92

DECEMBER 12, 1976, Experiment C-1
CHAMBER CORRECTION: .966 .971 .864 1.032 1.042 1.047
EXPERIMENT CORRECTION: 0.50840

0 mM	1 mM	2 mM	3 mM	4 mM	6 mM
81.20	136.34	139.40	193.29	296.79	325.00
71.88	36.38	181.44	280.65	300.74	531.59
97.96	182.59	226.91	339.81	409.70	496.24
69.76	203.14	169.94	305.27	316.32	500.78
96.06	145.38	170.18	286.31	409.18	617.13
83.37	139.09	177.57	281.07	346.55	494.26

DECEMBER 19, 1976, Experiment B-1
CHAMBER CORRECTION: 1.053 1.011 1.007 .988 .928 1.009
EXPERIMENT CORRECTION: 0.82780

0 mM	1 mM	2 mM	4 mM	8 mM	16 mM
155.30	191.24	219.15	242.22	203.50	269.09
162.70	167.05	121.36	291.27	299.38	122.50
129.74	174.39	280.64	239.98	292.80	304.93
173.82	137.29	155.23	187.42	209.35	245.65
182.82	206.43	196.23	239.66	229.58	380.67
197.99	154.52	182.14	229.32	270.31	369.64
167.06	171.82	192.46	238.31	250.82	282.08

DECEMBER 27, 1976, Experiment S-5
CHAMBER CORRECTION: 1.049 1.019 1.059 1.01 .923 .938
EXPERIMENT CORRECTION: 0.85790

0.04%	0.11%	0.23%	0.39%	1.05%	2.26%
165.86	196.22	158.66	175.55	121.71	112.80
112.76	135.90	146.03	131.93	108.68	103.54
101.34	113.24	138.56	75.01	104.78	114.34
117.23	130.81	128.17	127.07	160.91	93.65
150.75	149.70	215.08	148.93	177.84	141.76
161.64	157.41	250.24	138.16	121.52	128.24
134.93	147.21	172.79	132.77	132.57	115.72

DECEMBER 28, 1976, Experiment S-6
CHAMBER CORRECTION: 1 1.034 .945 1.002 1.014 1.005
EXPERIMENT CORRECTION: 0.81067

0.04%	0.11%	0.23%	0.39%	1.05%	2.26%
89.92	17.22	106.60	92.59	71.72	73.80
32.02	102.91	54.69	132.45	65.96	88.41
92.02	140.66	136.01	189.87	114.31	154.45
67.15	155.49	88.69	132.65	116.23	80.08
73.98	147.85	110.00	94.79	172.01	89.09
136.08	160.90	81.63	151.21	121.02	108.83
81.86	120.84	96.27	132.26	110.21	99.11

DECEMBER 29, 1976, Experiment B-2
CHAMBER CORRECTION: .993 .928 1.03 .954 .987 1.112
EXPERIMENT CORRECTION: 0.50550

0 mM	2 mM	3 mM	4 mM	8 mM	16 mM
98.07	144.92	133.44	214.46	186.56	418.31
124.18	177.01	180.36	300.54	427.66	451.42
102.37	182.76	177.69	234.81	351.28	446.79
97.63	176.09	170.29	146.56	264.98	344.45
155.45	206.89	246.74	181.58	248.42	431.29
116.94	193.57	230.55	295.96	301.70	519.08
115.77	180.21	189.84	228.98	296.76	435.22

DECEMBER 30, 1976, Experiment S-7
CHAMBER CORRECTION: 1.013 1.023 .987 1.013 .965 .993
EXPERIMENT CORRECTION: 0.80790

0.04%	0.23%	0.39%	1.05%	2.26%	5.45%
82.84	67.48	69.26	63.75	88.63	51.33
96.27	129.47	104.25	99.36	90.38	48.92
104.85	118.21	124.33	144.45	81.56	53.92
94.41	120.40	99.94	162.64	131.00	38.07
94.95	136.49	121.23	73.62	97.70	59.01
99.30	126.79	170.62	159.93	151.01	58.79
95.44	116.47	114.94	117.29	106.71	51.68

JANUARY 23, 1977, Experiment H-2
CHAMBER CORRECTION: .983 1.016 1.022 .973 .995 1.01
EXPERIMENT CORRECTION: 0.66830

TRIS	HEPES	CONTROL	TRIS	HEPES	$NaHCO_3$
92.02	56.87	161.76	55.64	74.90	315.19
69.16	109.38	209.74	76.61	125.44	356.21
54.36	88.53	175.22	80.24	98.39	293.07
68.66	99.68	149.00	95.34	72.58	387.04
65.27	109.38	134.64	49.69	103.16	458.43
67.96	51.54	181.88	43.93	75.59	369.56
69.57	85.89	168.70	66.91	91.68	363.25

FEBRUARY 11, 1977, Experiment S-8
CHAMBER CORRECTION: 1.027 1.012 .993 .897 1.043 1.028
EXPERIMENT CORRECTION: 0.37910

0.04%	0.11%	0.26%	1.05%	2.26%	5.46%
81.59	133.84	165.60	71.17	115.76	62.29
98.86	116.05	125.43	130.55	83.89	61.57
147.48	126.15	156.60	151.05	145.42	107.99
98.35	129.16	196.48	122.49	142.99	98.81
140.06	144.93	163.61	141.29	155.18	77.67
136.29	152.61	155.44	145.99	121.02	81.60
117.10	133.79	160.53	127.09	127.38	81.66

FEBRUARY 14, 1977, Experiment S-9
CHAMBER CORRECTION: .974 1.029 1.011 1.016 1.025 .945
EXPERIMENT CORRECTION: 0.67237

0.04%	0.11%	0.26%	1.05%	2.26%	5.46%
102.65	138.45	154.35	164.49	146.11	76.49
142.67	160.14	175.51	146.09	113.35	71.12
126.22	168.88	166.35	163.14	145.43	87.92
155.58	216.44	181.86	187.90	167.93	83.79
152.40	156.41	219.68	158.31	161.81	121.82
128.37	156.30	197.85	176.58	97.40	75.93
134.65	166.10	182.60	166.08	138.67	86.18

MARCH 21, 1977, Experiment S-10
CHAMBER CORRECTION: 1.044 1.031 .99 1 .986 .948
EXPERIMENT CORRECTION: 0.61080

0.04%	0.11%	0.26%	1.05%	2.26%	5.46%
153.73	169.18	163.45	140.09	118.09	74.72
155.18	182.65	290.32	192.58	141.97	127.57
142.90	187.73	165.06	162.52	127.97	84.23
113.38	198.44	149.20	118.00	163.43	93.31
228.08	295.62	310.85	243.27	179.31	74.04
149.90	184.68	193.92	135.67	132.29	126.24
157.20	203.05	212.13	165.35	143.84	96.68